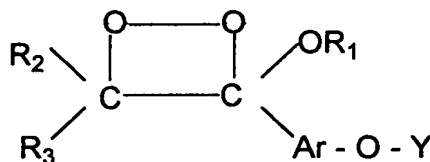




## Claims

1. A chemiluminescence detection system, comprising:
  - (a). An enzyme triggerable stable chemiluminescent 1,2-dioxetane
  - (b). An enhancer
  - (c). An enzyme diluent or stabilizer
2. The system of claim 1, wherein the 1,2-dioxetane, hereof corresponds to the formula: —



Wherein  $R_2$  and  $R_3$  are each organic groups which when combined together form an unsaturated organic group or when uncombined at least one of the  $R_2$  and  $R_3$  contains an unsaturated carbon double bond or triple bond and Y is an enzyme cleavable group and  $R_1$  is either a substituted or unsubstituted aryl, alkyl, aralkyl, alkaryl, alkene or alkyne

3. The system of claim 2 wherein:
  - (1) when Ar-O-Y and OR join together to give an aryl group substituted with an O-Y group to form a stable 1,2-dioxetane intermediate which is triggerable to form an unstable intermediate oxide,  $R_2$  and  $R_3$  either form (a)  which is either cyclic, polycyclic or a spiro-fused ring containing at least one carbon-carbon double bond or carbon-carbon triple bond in the ring or side chain with or without hetero atoms, or (b)  which is either cyclic, polycyclic or a spiro-fused ring containing a substituted or unsubstituted fused aromatic ring or substituted or unsubstituted aromatic ring attached by linker arms;

(2) when Ar-O-Y and OR<sub>1</sub> do not join together

Ar is an aryl which may be phenyl, substituted phenyl, naphthyl, substituted naphthyl, anthryl, substituted anthryl and the like as well as a nonaromatic fluorescent or nonfluorescent group; Y is hydrogen, alkyl, acetate, t-butyldimethylsilyl or an enzyme or a group of enzymes cleavable group, or an antibody cleavable group; R<sub>1</sub> is selected from the group consisting of alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl, cycloheteroalkyl, alkyletheralkyl, alkyletheraryl, alkyl(etheralkyl)<sub>2</sub>, alkyl(etheralkyl)<sub>3</sub>, alkyletherhaloalkyl, alkyl(etherhaloalkyl)<sub>2</sub>, alkylalkene, alkylalkyne, arylalkene, arylalkyne, linear or branched halogenated alkyl, alkylalcohol, alkyl nitrile, alkylamine, alkylacid or an inorganic salt thereof, haloalkylalcohol, haloalkyl nitrile, haloalkylamine, haloalkylacid or an inorganic salt, thereof, linker-flourescent molecule, linker-antibodies, linker-antigen, linker-biotin, linker-avidin, linker- protein, linker-carbohydrates or linker-lipids; R<sub>2</sub> and R<sub>3</sub> form either (i)  $\bigcirc^{\text{IR}}$  which is cyclic, polycyclic or a spiro-fused ring containing at least one carbon-carbon double bond or cabon-carbon triple bond in the ring or side chain with or without heteroatoms, or (ii)  $\bigcirc^{\text{Ar}}$ , which is cyclic, polycyclic or a spiro-fused ring containing substituted or unsubstituted fused aromatic ring or substituted or unsubstituted aromatic rings attached by linker arms; wherein or (iii)  $\bigcirc^{\text{R}}$ , which is a cyclic or polycyclic alkyl group or spiro-fused ring with or without substitution or (iv) are branched alkyl and cycloalkylgroups containing 3 to 8 carbon atoms which can contain halogens and hetero atoms in the ring or side chain thereof.

4. The system of claim 2 wherein the 1,2 dioxetane is [(4-methoxy)-4-(3-phosphoryloxy-4-chlorophenyl)] spiro [1,2 dioxetane-3,13'-tricyclo[7,3,1,0<sup>2,7</sup>] tridec-2,7-ene], disodium salt.
5. The system of claim 1 wherein the enhancer is selected from the group consisting of an ammonium or a phosphonium polymeric salt and mixtures thereof
6. The system of claim 5 wherein the polymer salt comprises a fluorescent molecule.
7. The system of claim 5 wherein the enhancer is a water-soluble polymeric compound prepared by reacting polyvinylbenzyl chloride with either tributylphosphine or tributylamine to form the phosphonium salt or ammonium salt, respectively.
8. The system of claim 5, wherein the enhancer is partially water-soluble, the enhancer being prepared by the reaction of polyvinyl benzyl chloride with either a 4:1 weight ratio mixture of (a) trioctylphosphine and tributylphosphine or (b) trioctylamine and tributylamine.
9. The system of claim 5 wherein the enhancer is a water-insoluble polymeric compound prepared by reacting polyvinylbenzyl chloride with either trioctylphosphine or trioctylamine to form the phosphonium salt or ammonium salt, respectively
10. The system of claim 9 wherein the polymer comprises a fluorescent molecule.

11. The system of claim 1 wherein the enzyme diluent comprises an aqueous mixture of:

- (a) a metal halide,
- (b) an alcohol,
- (c) an amine-based salt,
- (d) a blood or plant protein or mixtures thereof, and

wherein the diluent has a pH of from about pH 7 to about pH 10, the diluent corresponding to blood components, and further wherein the system is enzyme triggerable at single molecule detection levels.

12. A method of chemiluminescence detection, which comprises; contacting the system of claim 1 with an enzyme selected from the group consisting of alkaline phosphatase, beta galactosidase, and cholinesterase()

13. A method for enhancing the chemiluminescence of a 1,2-dioxetane which comprises: admixing with the dioxetane and enhancer which is the reaction product of a polyvinyl benzyl chloride and either a phosphine, an amine or mixtures thereof to form a polymeric salt()

14. The method of claim 13 wherein the polymer comprises a fluorescent molecule()

15. The system of claim 13 wherein the enhancer is a water-soluble(?)

16. A polymeric compound prepared by reacting polyvinylbenzyl chloride with either tributylphosphine or tributylamine to form the phosphonium salt or ammonium salt, respectively.

17. The system of claim 13, wherein the enhancer is a partially water-soluble, the enhancer being prepared by the reaction of polyvinyl benzyl chloride with either a weight ratio mixture(4:1) of either; (a) trioctylphosphine and tributylphosphine or (b) trioctylamine and tributylamine.

18. The system of claim 13 wherein the enhancer is a water-insoluble polymeric compound prepared by reacting polyvinylbenzyl chloride with either trioctylphosphine or trioctylamine to form the phosphonium salt or ammonium salt, respectively

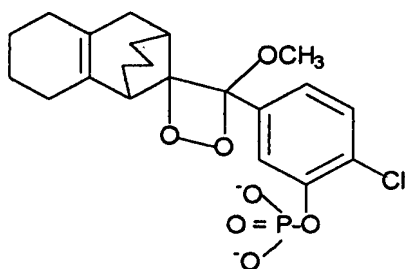
19. An enzyme diluent for an enzyme triggerable stable 1,2-dioxetane which comprises

- (a) a metal halide,
- (b) an alcohol,
- (c) an amine-based salt,
- (d) a blood protein or a plant protein or mixtures thereof,

20. The enzyme diluent of claim 18 wherein:

- (a) the metal halide is selected from the group consisting of sodium chloride, potassium chloride, zinc chloride, magnesium chloride and mixtures thereof,
- (b) the alcohol is selected from the group consisting of ethyl alcohol, propyl alcohols, butyl alcohols, ethylene glycol, ethylene glycol methyl ether, glycerol and mixtures thereof,
- (c) the amine based salt is selected from the group consisting tris(hydroxymethyl-aminomethane, diethanolamine, triethanolamine, 2-amino-2-methyl-1-propanol and mixtures thereof,
- (d) the blood protein is from human or an animal sources or from a plant source

21. The system of claim 3 wherein the chemiluminescent 1,2-dioxetane has the following structure



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